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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C08B	A2	(11) International Publication Number: WO 97/34932
		(43) International Publication Date: 25 September 1997 (25.09.97)

(21) International Application Number: PCT/GB97/00788

(22) International Filing Date: 20 March 1997 (20.03.97)

(30) Priority Data:
9605857.3 20 March 1996 (20.03.96) GB

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: COMPOSITIONS CONTAINING STARCH EXCIPIENTS

(57) Abstract

Compositions containing starch and its components as excipients for use as delayed, controlled and targeted release formulations. It is possible to tailor the form of release from the compositions according to the nature of the starch excipient.

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COMPOSITIONS CONTAINING STARCH EXCIPIENTS

The present invention is concerned with compositions containing mechanically damaged and pre-gelatinised starch and components thereof.

The use of starch in both food and non-food related applications has been widely documented and is summarised by JJM Swinkels in a series of publications produced by Avebe b.a. of the Netherlands entitled "Industrial Starch Chemistry", "Differences between Commercial and Native Starches" and "Starch Terminology".

Potato starch has been used in the preparation of adhesives and paper coatings. Maize starch has been used in the preparation of home-cooked puddings having a smooth, short texture and is also used in the preparation of a wide variety of other foodstuffs. WO 94/10993 discloses the use of both hydrolysed and pre-gelatinised potato starch as a binder in quantities of between 0.5 and 2.0% by weight to facilitate both the processing and tableting of the dosage form and to ensure advantageous dissolution properties including rapid disintegration and release of the active agent.

Sources of starch include cereal grains such as maize, waxy maize, wheat, rice and waxy rice; tubers such as potato; roots such as tapioca, sweet potato and arrowroot and the pith of the sago palm. Starch is extracted in the form of granules using processes which are well documented.

The two major components of starch are amylose and amylopectin; both are condensation polymers of glucose. Amylose is a linear polysaccharide derived from repeating anhydroglucose units connected through alpha-1,4-glycosyl linkages. Amylopectin is a branched chain polysaccharide having repeating anhydroglucose units connected by alpha-1,4-glycosyl linkages and in addition alpha-1,6-glucosyl linkages at selected sites along the alpha-1,4-chain thereby generating a branch point. The relative amounts of amylose and amylopectin in any starch granule and the degree of polymerisation thereof depends upon the source of the starch. The degree of polymerisation of the amylose and amylopectin in the native starch granule can be determined using Gel Permeation Chromatography, J Karkalas and RF Tester, J Cereal Science, 15 (1992) 175 - 180. For example potato starch contains 21% amylose and maize starch contains 28% amylose. In general the amount of amylopectin present is generally greater than the amount of amylose.

During the development of starch granules deposition of a minute amount of an insoluble polysaccharide is formed which acts as a nucleus (known as the hilum) from which starch molecular chains grow. During the initial stages of granule formation more amylopectin is formed. As the granule formation progresses the proportion of amylose formed increases.

The effect of environmental conditions on the composition of the starch granules has been documented, see for example RF Tester, JB South, WR Morrison and RP Ellis, J Cereal Science 13 (1991) 113 - 127 and RF Tester, WR Morrison, RH Ellis, JR Piggott, GR Batts, TR Wheeler, JIL Morison, P Hadley and DA Ledward, J Cereal Science, 22 (1995) 63 - 71.

Amylopectin is believed to be responsible for the crystalline regions in the starch granule. The basic unit of the ordered material contributing to the crystallinity is a double helix formed between the external parts of adjacent A and B chains of the amylopectin. All but one or two glycosyl residues of these chains next to the alpha-1,6-branch point are able to form a helix. It is thought (WP Morrison, RF Tester and MJ Gidley, J Cereal Sci., 19 (1994) 209 - 217) that parallel helices can assemble into radially-oriented clusters giving rise to a shell of ordered crystalline material when there are sufficient extending clusters in a curving plane that are close enough for continuity. The crystalline regions are responsible for the birefringent properties of the granule.

WR Morrison, RF Tester and MJ Gidley, J Cereal Science, 19 (1994) 209 - 217, have proposed a model for a starch granule which comprises essentially continuous concentric shells of crystalline, ordered amylopectin separated by broad bands of amorphous amylose and amorphous amylopectin. The amorphous regions derived from amylopectin comprise the narrow alpha-1,6-branched regions derived from the B1 and B2 chains between the concentric double helical clusters of amylopectin molecules. These regions are also thought to traverse broad amorphous regions between the concentric shells that are also occupied by amorphous amylose.

In addition to amylose and amylopectin, starch granules may also contain additional components such as proteins and lipids. The lipids may be present as inclusion complexes wherein they are complexed with a helical amylose molecule. Inclusion

complexes are most commonly found in cereal starches as these contain a higher proportion of lipids compared with potato and tapioca starch.

Amylose inclusion complexes of stearic, oleic, linoleic and linolenic acids have been documented by J Karkalas, S Ma, WR Morrison and RA Pethrick, Carbohydrate Research, 268 (1995) 233 - 247, their preparation requiring the precipitation of the complex from a mixture of fatty acid and amylose. The conditions employed in the formation of these inclusion complexes is critical. Dissociation of the complexes occurs at higher temperatures or under conditions of high shear force.

Native starch granules are insoluble in cold water and do not tend to swell due to the presence of the crystalline concentric shells of amylopectin. Any swelling that does occur is due to absorption of water by the amorphous amylose and amylopectin regions present between the shells.

Gelatinisation and swelling of the granule occurs on continued heating in water. The process of gelatinisation is well documented and is characterised by the gelatinisation endotherm which can be followed using differential scanning calorimetry (DSC). Gelatinisation is initially characterised by the disordering of the amylopectin crystallites and loss of birefringence around an onset temperature T_o. Further heating results in the dissociation of the non-birefringent amylopectin helices at a temperature between T_o and a peak temperature T_p. Above T_p it is postulated that the external chains have a restricted semi-random conformation due to the swollen nature of the molecule. Finally disaggregation of the starch granules occurs to give starch solutions at the conclusion temperature T_c. The onset, peak and conclusion gelatinisation temperatures are dependant on the source of starch (RF Tester and WR Morrison, Cereal Chem., 67(6), 551 - 557).

Heating the starch granule destroys the integrity of the crystallites thereby exposing the amylose and amylopectin molecules to attack by the water. Starch comprises polyhydroxy compounds which hydrate when heated in water. As the starch molecules hydrate they swell and immobilise much of the water present. The characteristic swelling properties of starch are mostly exhibited at temperatures above the gelatinisation range.

The degree to which starches swell depends upon the source of starch. RF Tester and WR Morrison, Cereal Chem., 67(6), 551 - 557; RF Tester and WR Morrison, Cereal Chem., 67(6), 558 - 563 and RF Tester and WR Morrison, Cereal Chem., 69(6), 654 - 658 have observed that starches with little or no amylose swell to the greatest extent. The presence of amylose lipid inclusion complexes tends to inhibit swelling.

It has frequently been found that native starch isolated from a particular source does not necessarily provide the best product for a certain applications or processes and for this reason modifications are made to provide starch granules having properties better suited to those applications. Physical, chemical and enzymic modifications have all been used for this purpose and are well documented. In general the modified starches thereby prepared retain characteristics of the native starches from which they are derived.

Chemical modifications usually give rise to low viscosity starches subjecting the native starch to a treatment which causes some of the glucosidic bonds to randomly rupture resulting in the formation of lower molecular weight fragments which tend to have a liner structure. Starch treated in this way is more easily gelatinised but tends to swell less than gelatinised native starch granules.

Enzymic modifications involve depolymerisation of the starch by the action of amylases giving starches with reduced viscosities. Each amylase has its own pattern of hydrolysis resulting in molecules of reduced length or having fewer branches. Alpha-amylases attack the starch molecules by hydrolysing the 1,4-alpha-glucosidic bonds. Beta-amylases also attack the 1,4-alpha-glucosidic bonds but do so in such a way that maltose units are successively released from the non-reducing ends of the starch chains. Glucoamylase converts starch almost completely to D-glucose by the successive removal of D-glucose units from the non-reducing ends of 1,4-linked starch chains and by hydrolysing the 1,6-branching linkages.

Physical means of modification usually require precooking (pre-gelatinising) the native starch granule to give a product which is swellable in cold liquid without cooking. Although the integrity of pre-gelatinised starch granule is retained they are characterised by a loss of crystalline order and may be used to improve the texture, processing properties and physical characteristics of the products in which they are included.

Precooked starches find particular application in the preparation of convenience foods, wallpaper adhesives and drilling mud.

Other forms of modification include mechanical damage which occurs as a result the continued grinding or milling of the granules. This form of damage is considered to be an undesirable modification. The changes which occur to the native starch granule as a result of milling depend upon the extent of the damage imposed. Starch granules having a low to medium extent of damage are characterised by the presence of stress fractures and cracks in the body and at the surface of the granule together with some loss in crystallinity. Larger amounts of damage result in fragmentation of the starch granule, a substantial loss in crystallinity and the release of gel forming and water soluble low molecular weight material derived from amylopectin when hydrated. The changes and properties associated with mechanically damaged starch are well documented, WR Morrison, RF Tester and MJ Gidley in J Cereal Science, 19 (1994) 209 - 217; RF Tester, WR Morrison, MJ Gidley, M Kirkland and J Karkalas, J Cereal Science, 20 (1994) 59 - 67; WR Morrison and RF Tester, J Cereal Science, 20 (1994) 69 - 77; and RF Tester and WR Morrison, J Cereal Science 20 (1994) 175 - 181. As a result of these changes mechanically damaged starch spontaneously swells when placed in water.

The starch used in the preparation of foodstuffs is usually processed according to strict processing parameters to avoid mechanical damage. Mechanically damaged starch is considered to be unsuitable for use in the preparation of foodstuffs. Foodstuff preparation generally requires the use of starches with both controllable and predictable swelling characteristics. The type of damage associated with mechanically damaged starch granules is quite different to the changes brought about by chemical or enzymic modifications. In particular, mechanically damaged starch is characterised, in contrast to chemically and enzymically modified starch, by the production of amorphous gel forming material as well as water soluble low molecular weight material, see WR Morrison and RF Tester, J Cereal Science, 20 (1994) 69 - 77. The inability to control the swelling and processing characteristics of mechanically damaged starches makes them unsuitable for use in the preparation of foodstuffs. In addition the properties of damaged starches tend to increase the complexity of processing and result in foodstuffs having an inferior texture.

Starch has been widely used in the preparation of pharmaceutical and non-pharmaceutical compositions. Starch is generally considered to be a good binder due to its adhesive properties and is often introduced into the formulation mixture as an aqueous solution in which the crystallinity of the granule has been lost. Pharmaceutical formulations containing starch in quantities of between 0.5 and 2% have been prepared in WO 94/10993. These formulations were observed to have good tableting properties and to undergo rapid disintegration. Formulations containing starch are not generally considered to be suitable for controlled, delayed or targeted release unless coatings are also used as they either rapidly disintegrate or lose their integrity due to attack on the amylopectin by amylase enzymes of the small intestine.

The composition of a controlled, delayed or targeted release formulation is generally dictated by the type of release required. Compositions containing amylose as a coating or an excipient have been used for delivery of active material to the colon and those containing ethylcellulose for delivery to the stomach. Materials used in the preparation of delayed release formulations are usually biocompatible but are not necessarily bio-degradable. It is therefore appreciated that it is not possible to use excipients to effect controlled, delayed and targeted release. At present different excipients will be selected depending upon the type of release required. The materials may be expensive and pose a problem for disposal if they are not biodegradable.

There is a need for an inexpensive, biodegradable excipient whose properties can be varied according to the nature of the release required. The present invention addresses this problem.

The ability of starch containing compositions to control the nature and type of release over a wide range of conditions has not been previously recognised. The present inventor has found that by using mechanically damaged starch, pre-gelatinised starch and inclusion complexes of alpha-glucans including starch as herein described or mixtures thereof, it is possible to prepare compositions from a single excipient having a variety of release characteristics.

A first aspect of the invention provides compositions comprising mechanically damaged starch granules and one or more active compounds. The term "damaged starch granules" includes whole starch granules having little or no damage, granule fragments,

gel forming material and low molecular weight water soluble material formed as a result of the physical damage. The starch may be damaged by any physical means, for example by grinding, pounding and milling. Preferably the starch granules are damaged by milling, especially ball milling as this has been found to give reproducible and consistent results. The compositions will have many industrial applications such as absorption of spillages in food and non-food applications as well as the absorption of blood in dressings.

Highly damaged starch granules resemble a powder and comprise mainly granule fragments; they are characterised by a loss in integrity and little or no associated crystalline structure. Less damaged starch granules include granule fragments, gel forming material and water soluble low molecular weight material formed as a result of the physical damage. The extent of damage imposed upon the granules depends upon the composition of the native granule, its size and source and can readily be determined by the method of Karkalas; J Karkalas, RF Tester and WR Morrison, J Cereal Science, 16 (1992) 237 - 2551. The nature of the products produced by mechanical damage has been herein before discussed and can be determined using Gel Permeation Chromatography (GPC) see J Karkalas and RF Tester, J Cereal Science, 15 (1992) 175 - 180. Rice granules having a higher amount of amylopectin and a small granule size are more susceptible to damage than maize and pea starches which have a higher amylose content and a larger granule size. The extent and effect of mechanical damage on wheat and maize starches is documented: WR Morrison, RF Tester and MJ Gidley, J Cereal Science, 19 (1994) 209 - 217; WR Morrison and RF Tester, J Cereal Science, 20 (1994) 69 - 77 and RF Tester and WR Morrison, J Cereal Science, 20 (1994) 175 - 181. Less damaged starch granules retain some crystallinity, do not swell as much and give rise to a little gel forming and water soluble material when hydrated. More extensively damaged starches retain little of their native crystallinity, spontaneously swell in water and release a larger amount of gel forming and water soluble material in water.

Any active material may be used, the proportions of starch and active material depending upon requirements. Typically the compositions should contain between 5 and 95% active material and between 95 and 5% damaged starch. The compositions of the invention may be used for both pharmaceutical, agricultural and industrial purposes.

For example, the compositions could be used for controlled release of pesticide, the slow release of dyes or targeted release of drugs. The intended function of the active material would determine the choice of mechanically damaged starch as excipient and the nature of the formulation. The pharmaceutically active compounds which may be usefully incorporated into the compositions of the invention include all those which may be formulated as tablets by a wet or dry granulation process. The range of active compounds is thereby a wide one and indeed any active compound which is not significantly degraded upon formulation may be used.

Examples of active materials which may be used include β-blockers such as atenolol and metroprolol; calcium antagonists such as nifedipine and nitrendipine, ACE inhibitors such as enalapril and captopril, β2 agonists such as salbutamol and terbutaline, hormones, for example androgenic, estrogenic and progestational hormones, hypoglycaemic agents, contraceptives, nutritional agents, peptides and proteins, nitrates such as isorbide dinitrate, mononitrate and GTN; xanthines such as theophylline; NSAIDs such as piroxicam, diclonfenac, brufen and ibuprofen; benzodiazepines such as triazolam; antivirals such as acyclovir and zidovudine; cephalosporins such as cefaclor; salicylates such as 5-amino salicylic acid and preparations containing analgesics such as aspirin.

The active material of the composition may comprise together with the damaged starch granules a matrix formulation or it may be in a form in which it is more intimately associated with the damaged starch granule. Forms of intimate association envisaged include adsorption and absorption of the active compound or material by the damaged starch granule or fragments thereof or by entrapment within and at the surface thereof. Without wishing to limit the scope of the invention it is believed that the form of association will depend upon the extent to which the starch granule is damaged as well as the nature of the active ingredient. For example starch granules having a low amount of damage may trap active compounds within the cracks and fissures formed in the granule. At a higher level of damage more granule fragments are produced and the active compound may become associated through adsorption and absorption with these fragments or by entrapment within the gel forming material derived from the

amylopectin. The form of association may depend upon the conditions used to prepare the compositions; pH, solvents and ionic strength each have an influence.

The choice of formulation influences the release characteristics obtained. For example if compositions having a range of immediate release characteristics are required the damaged starch compositions may be formulated as capsules. The choice of capsule will depend upon the target site for the release. If immediate release upon ingestion is required a capsule whose integrity is destroyed during this step should be used, for example gelatin. If it is desired to target the active compound for immediate release at a site such as the colon capsules having enteric coatings are preferred. The level of mechanical damage of the starch will also affect the rate of release. Immediate release is favoured by using highly damaged starch with a low degree of crystallinity which is able to swell on contact with water, gastric, ileal or colonic juices.

The choice of damaged starch granules will also depend upon the granule composition. Crystalline material within starches comprising clusters of amylopectin double helices are not hydrolysed in the small intestine by amylases but may to some extent be hydrolysed by the action of microorganisms in the colon. Amorphous amylopectin on the other hand is readily hydrolysed by amylases in the small intestine. Similarly, amorphous amylose does not resist amylase hydrolysis; however, when amylose is retrograded (where double helices have formed) or when complexed with guest molecules (like lipids) it is much more resistant to hydrolysis. The ease of attack by the amylose enzymes depends upon the form in which the amylopectin is present; amorphous amylopectin is attacked more readily than crystalline amylopectin. Therefore it is possible, using damaged starch granules with a high amylose content to prepare a composition which is substantially resistant to degradation prior to entry into the colon. Conversely damaged starch granules having a higher amylopectin content are preferred for delivery of an active compound to the small intestine; starch granules having a low degree of damage and which retain a substantial degree of their native crystallinity are preferred for delayed release whereas the use of more extensively damaged starch granules is preferred when faster release characteristics are required.

Alternatively and in a preferred embodiment of the first aspect of the invention, damaged starch may be formulated, together with one or more active ingredients, into

a tablet formulation. As before, the composition and the level of damage of the starch granules affects the type of release observed. Surprisingly, tablets formulated using starch having between 80 and 100% damage were observed to undergo slow disintegration, remaining intact for periods exceeding 3 hours, thereby delaying release of the active ingredient. In contrast, tablets formulated using undamaged native starch undergo immediate disintegration. Without wishing to limit the scope of the invention it is believed that the slow disintegration and delayed release properties associated with tablets formulated using mechanically damaged starch granules are due to the rapid swelling of the surface granules in water which creates a barrier thereby inhibiting the ingress of water into the body of the tablet and the escape of the active ingredient therefrom. The nature of the release of active compounds from tablets thus formed may be controlled by the choice of damaged starch which has the appropriate swelling characteristics; damaged granules which swell significantly inhibit disintegration to a greater extent than less damaged granules. The properties of mechanically damaged starch granules and the factors influencing their ability to swell when hydrated are well documented and the reader is referred to WR Morrison, RF Tester and MJ Gidley, J Cereal Science, 19, (1994) 209 - 217; RF Tester WR Morrison, MJ Gidley, M Kirkland and J Karkalas, J Cereal Science, 20 (1994) 59 - 67; WR Morrison and RF Tester, J Cereal Science 20 (1994) 69 - 77; and RF Tester and WR Morrison, J Cereal Science 20 (1994) 175 - 181.

It may be desirable to include further excipients or auxiliaries in the compositions in order to promote or retard disintegration of the composition. Auxiliaries which promote disintegration are typically hydrophilic, for example, cellulose polymers such as carboxymethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, methylcellulose, sodium carboxymethylcellulose, galactomannose, sodium alginate, kaolin, bentonite and talc. Tableted compositions comprising mechanically damaged starch and Avicel have been found to disintegrate in a particularly advantageous fashion. Hydrophobic auxiliaries tend to retard disintegration. Tablets comprising Avicel, damaged starch and aspirin as an active ingredient are of especial interest. Examples of hydrophobic auxiliaries include polyethylene, polyvinylchloride, methacrylate-methacrylate co-polymer, fatty acid esters, triglycerides and carnuba wax.

The compositions of the invention may be formulated for a variety of applications depending upon the release characteristics required. It is envisaged that the compositions of the invention are suitable for delayed, controlled and targeted release. Modes of release include oral, rectal, vaginal, subcutaneous, intravenous, by inhalation and by topical administration. Examples of formulations include capsules, tablets, pessaries, dragees, patches, powders, lotions, injectible formulations, sprays and implants.

A second aspect of the invention provides a method for the preparation of the composition comprising admixing mechanically damaged starch granules, one or more active ingredients and optionally one or more auxiliaries. The compositions should be formed at temperatures below the disintegration temperature of the active compound or material. The composition may be formed at temperatures between 0 and 150°C, preferably between 15 and 80°C and especially between 25 and 40°C.

The auxiliaries employed will depend upon the desired release properties as well as the nature of starch and the active compound. It may be desirable to use a small amount of a physiologically acceptable solvent, suitably water, during formulation of a composition for human or animal use in order to facilitate either formulation or more intimate association of the active compound with the damaged starch granules. The amount of water will depend upon the damaged starch granules used and should be sufficient to cause the active compound to enter or form an association with the damaged starch granules without causing the granules to swell appreciably. The swelling properties of mechanically damaged starches are well documented in the references herein above cited and a skilled person will, in the light of the present disclosure, be able to prepare compounds according to the invention without undue experimentation.

Other auxiliaries may suitably be used to facilitate or inhibit disintegration of the composition or as lubricants for the formulation step. Lubricants include fatty acid salts and their esters, for example magnesium stearate or sodium palmitate or wax.

A third aspect of the invention provides the use of damaged starch granules as excipients. As indicated above the choice of damaged starch will depend upon the desired properties of the composition. The factors influencing that choice are well documented in the light of the present invention and the reader is referred to the aforementioned papers by Tester et al.

The invention also provides, in a fourth aspect, damaged starch for use in therapy.

It is also possible to control the release characteristics of a composition over a range of conditions by using partially gelatinised native starch granules instead of or in addition to mechanically damaged starch granules as used above. The use of partially gelatinised starch granules may be preferred, for example, where more controlled swelling characteristics are required or where it is desirable to retain some of the release characteristics of individual granules.

A fifth aspect of the invention provides a composition comprising partially gelatinised starch granules and an active compound wherein the active compound is entrapped within the partially gelatinised starch granule. Active compounds entrapped within the starch granules are known as guest molecules. Any active compound or material suitable for inclusion into compositions according to the first aspect of the invention is also considered to be suitable for formation of a gel entrapped composition. Upon drying the guest molecule becomes trapped within the starch granule. The granules may be ground into a powder for further formulation if required and used as molecular delivery systems. Compositions according to the fifth aspect of the invention may be used to selectively deliver guest molecules, for example vitamins and other therapeutic agents, to specific parts of the gastro-intestinal tract. In the small intestine the amylases will digest the amorphous starch and a more extensively gelatinised and swollen starch would be required for delivery in this region. In the colon, the indigenous microflora have the potential to metabolise resistant starches; in this environment a less extensively gelatinised and swollen starch would be appropriate for drug delivery. Drugs released from the matrix will not readily irritate the mouth or stomach mucosa upon ingestion or be modified by the stomach acid. In addition the taste of the active ingredient is disguised and the potential for oxidation is reduced.

The release characteristics of the compositions according to the fifth aspect of the invention depend upon the extent of gelatinisation of the starch granule. Partially gelatinised native starch granules containing guest molecules are not able to release the entrapped guest molecule as readily as compared with granules coated with the guest

molecule unless the granule is more extensively gelatinised and swollen, hydrolysed or mechanically damaged.

Native, modified or damaged starch granules may be used to form the compositions of the fifth aspect of the invention. Native starches may be gelatinised by heating in water; the extent of gelatinisation may be limited by controlling the temperature and the amount of water present. The gelatinisation can be monitored using Differential Scanning Calorimetry (DSC). Heating the starch granule at temperatures between the onset (T_O) and peak (T_P) temperatures of the gelatinisation endotherm leads to progressive loss in the crystalline nature of the starch granule rendering it more susceptible to attack by amylase enzymes and causing it to swell. Without wishing to limit the scope of the invention it is believed that the disruption in the crystalline nature facilitates entry by the guest molecule into the starch granule by diffusion where it becomes trapped. Damaged starches may be gelatinised by placing in cold water; the extent of gelatinisation may be limited by controlling the amount of water present.

The choice of starch and the degree of gelatinisation will depend upon the desired release characteristics of the composition. Starches with a high lipid complexed or retrograded α-glucan content and/or high degree of crystallinity will be resistant to hydrolysis by the amylases in the small intestine but will to some extent be susceptible to fermentation by the microflora of the colon. Starches having a higher proportion of amorphous amylopectin and/or a lower degree of crystallinity will be more suitable for the preparation of compositions suitable for release in the small intestine. Extensively gelatinised starch granules therefore tend to release their guest molecules more readily than those less extensively gelatinised. Starch granules with a high lipid content also tend to swell less. The factors influencing the degree to which starch granules swell is well documented; see for example RF Tester and WR Morrison, Cereal Chem., 67(6), (1990), 551 - 557; RF Tester and WR Morrison, Cereal Chem., 67(6), (1990), 558 - 563; RF Tester and WR Morrison, Cereal Chem., 69(6), (1992), 654 - 658; RF Tester and J Karkalas, Cereal Chem., 73(2), (1996) and WR Morrison, RF Tester, CE Snape, R Law and MJ Gidley, Cereal Chem., 70(4), (1993), 385 - 391.

In some circumstances it may be desirable to anneal the partially gelatinised gel entrapped guest molecule containing compositions. This facilitates re-ordering of the

previously disordered amylopectin chains within the starch granule and further inhibits the release of active material therefrom.

The compositions according to the fifth aspect of the invention can be used to selectively deliver guest molecules to specific parts of the gastro-intestinal tract. They may also be used for a wide range of industrial and agricultural purposes, for example the slow release of chemicals, food components, drugs, dyes, fertilisers, lipids and proteins.

A sixth aspect of the invention provides a method for the preparation of compositions according to the fifth aspect of the invention comprising admixing a solution of partially gelatinised starch granules with one or more active materials. The degree to which the starch granules are gelatinised may be controlled and can be followed using DSC. The active material can be added before or after partial gelatinisation is effected. Active materials added before the gelatinisation step are preferably not temperature sensitive. Temperature sensitive active materials which are degraded at elevated temperatures should be added subsequent to gelatinisation.

The partial gelatinisation may take place in any suitable solvent. By suitable solvent it is to be understood as any solvent which permits the starch granule to swell. Water is the preferred solvent. There may be occasions when a mixture of solvents is necessary, for example, if it desired to entrap a guest molecule having a moderate degree of hydrophobicity. Suitable solvent mixtures include water and an alcohol, preferably ethanol and water and dimethylsulphoxide. If the composition is to be used for ingestion by a human or animal it the solvent of choice should be physiologically acceptable. The pH and ionic strength of the solvent system may also be varied as required in order to facilitate uptake by the granule of the active compound or to trap the granule in the most physiologically acceptable form. The solvent may have a pH of between 3 and 8, preferably 4 to 8 and especially 6 to 7. Preferably physiologically acceptable salts and the acids thereof are used to control the pH and ionic strength of the solution.

The composition is preferably dried subsequent to admixing but may be used "as prepared" if necessary. It may also be desirable to anneal the composition prior to use.

Annealing results in the ordering of the previously disordered amylopectin molecules rendering them more resistant to attack by the amylase enzymes of the small intestine thereby slowing the release of active material from the composition.

The compositions according to the fifth aspect of the invention may be formulated for oral, rectal, vaginal, sub-cutaneous, intraveneous, by inhalation or topical administration. Examples of formulations which can be used include dragees, tablets, capsules, powders, pessaries, patches, injectible compositions, sprays and ointments. Particular mention should be made of compositions containing, as active material, water soluble glutamine, ascorbate, palmitic acid and aspirin.

The release characteristics of the formulations may be further varied by the optional addition to the formulation of damaged or modified starch. Other auxiliaries as hereinbefore described may also be added to the formulation. Formulations comprising layers or regions of damaged and partially gelatinised starch are envisaged and can provide a variety of release characteristics which is of advantage if, for example, a particular effect is dependent upon the timed release of two or more active materials. The formulations may optionally be totally or partially coated if desired.

A seventh aspect of the invention provides the use of partially gelatinised starch granules comprising a guest molecule in the preparation of compositions for controlled, delayed and targeted release. The invention also provides the use of partially gelatinised starch granules comprising a guest molecule in the preparation of compositions for use in therapy.

The use of amylose containing controlled release compositions has been described in GB 2 230 243 B and WO 91/07949. In these compositions the amylose is used in pure form either as a coating or as part of the matrix formulation. The ability of the formulation to target the release at the colon is critically dependent upon the purity of the amylose. Retrograded or complexed amylose resists hydrolysis by the amylases within the small intestine but is more easily hydrolysed by the flora of the colon. Compositions containing amylopectin are unable to inhibit release of the active material before entry into the colon as the amylopectin susceptible to attack in the small intestine thereby destroying the integrity of the formulation and facilitating premature release of the active material. Matrix formulations comprising both amylose and amylopectin are unable to delay release prior

to entry into the colon as attack by the amylase enzymes on the matrix amylopectin creates pore from which the active material can escape; the active material is not protected from attack. There is therefore a further need for a carrier which is able to resist attack prior to entry into the colon.

Amylose-guest molecule (inclusion) complexes have been made with fatty acids and lipids containing fatty acid residues where the fatty acid residues are accommodated within the helical coils of the alpha-glucan, amylose; J Karkalas, S Ma, WR Morrison and RA Pethrick, Carbohydrate Research, 268 (1995) 233 - 247. The inclusion complexes have the potential of protecting the guest molecules complexed therewith due to the ability of the alpha-glucan to resist enzymatic attack, thereby providing further delayed release carriers. These helices also exist within native starch granules. These helices also exist within native starch granules and can be detected using DSC. Cyclodextrin-guest molecule complexes in which the guest molecule is a pharmaceutically active compound have been prepared (F Schierbaum and W Vorwerg, Starch, 48(1996) 422 - 426); cyclodextrin compounds are expensive to prepare and are also limited in their application due to the restricted size and number of conformations that the cyclodextrin can adopt. There is therefore a need for other species which can act as carriers for guest molecules and which are resistant to attack prior to entry into the colon.

Cyclodextrins have been used to complex pharmaceutically active material for targeted delivery. However, cyclodextrins are expensive to prepare and are limited in scope due to their restricted size and conformation. Amylose drug inclusion complexes have not been employed as a drug delivery system because of the relative difficulty of preparing the compounds. Amylose is a linear alpha glucan and is conformationally more flexible than cyclodextrins and has the potential to form inclusion complexes with a wider range of active materials.

An eighth aspect of the invention provides a composition comprising a linear alpha glucan and a guest molecule in which the guest molecule is complexed with helices of the alpha glucan. The alpha glucan can be a linear alpha-1,4 or alpha-1,6 glucan. The term linear is to be understood as including branched chain alpha glucans. Preferably the alpha glucan is a 1,4-alpha glucan, especially amylose or oligomers thereof. The alpha-glucan may be in a form in which it is substantially pure of impurities. Preferably the composition

comprises one or more alpha glucans. Starch granules containing the alpha-glucans amylose and amylopectin are especially preferred.

The inclusion complexes may be formed with isolated polysaccharide fractions within gels, mechanically damaged starch and native and/or modified starch granules. Retrograded starches and polymeric systems may be used where double helical polysaccharide chains entrap and in part complex with other chemical moieties. Any guest molecule may be introduced into the helices of the linear alpha glucan and the term guest molecule is to be understood as including one or more types of active species, bacteria, viruses, genes, gene fragments and hormones. Further examples of guest molecules of interest include those hereinbefore mentioned. Inclusion complexes containing β-carotene, folic acid, retinol acetate, aspirin and ibuprofen are particularly preferred. Aspirin has recently been suggested as being suitable for treatment of cancer of the colon and inclusion complexes containing aspirin are therefore valuable for targeting the delivery of aspirin to the colon. Fat soluble vitamins such as those derived from β-carotene are insoluble in water and susceptible to oxidation. Inclusion complexes of these fat soluble facilitate the provision of water soluble compositions that are not susceptible to oxidation. Linear alpha glucans offer advantages over the cyclodextrins of the prior art due to their ability to form complexes with a wide range of molecules of differing size and conformation. Amylose-drug inclusion complexes have not previously been employed as a drug delivery system due to the difficulty in producing the complexes.

The compositions of the invention comprising inclusion complexes form the basis for the provision of vitamin and drug delivery systems which can be used to protect the guest molecule by preventing oxidation thereof, modify solubility characteristics and mask bitter taste. The slow or controlled release of a guest molecule such as phenyl alanine may be used to control the release of this amino acid for phenylketoneurics, whilst slow release of anti-cancer drugs might be used to treat colonic cancer.

The compositions according to the eighth aspect of the invention may be formulated for oral, rectal, vaginal, sub-cutaneous, intraveneous, by inhalation or topical administration. Examples of formulations which can be used include dragees, tablets, capsules, powders, pessaries, patches, injectible compositions, sprays and ointments.

If the composition comprises one or alpha-glucans in the form of a starch granule

the starch granule may be a native starch granule, a chemically or enzymically modified starch granule or a mechanically damaged starch granule. The nature and extent of modification of the starch granule will alter the release characteristics of the composition. Without wishing to limit the scope of the invention it is believed that modification of the starch granule will influence the type of guest molecules that can be complexed and the nature of their subsequent release. It is also believed that the type of inclusion complexes formed by the gel-forming material of damaged starch granules will differ from those formed by the native fragments, thereby providing combined release characteristics.

A ninth aspect of the invention provides a method for the preparation of compositions according to the eighth aspect of the invention comprising admixing at a complexing temperature a solution of one or more alpha-glucans with one or more guest molecules. The solvents used for the preparation of solutions of both the alpha-glucan and the guest molecule depend upon the nature of these species.

If both the alpha-glucan and the guest molecule are water soluble, it is preferred to use water as a solvent. Other solvents which may be used include dimethylsulphoxide, ethanol, methanol or mixtures thereof. The pH and ionic strength of the solvent system may also be controlled. Preferably the pH of the solution is between pH 3 and 8, preferably between pH 5 and 7. If the compositions are to be used for human or animal use it is preferred to use physiologically acceptable solvents, salts and acids thereof.

The ratio of alpha-glucan to guest molecule will depend upon the nature of the guest molecule and the inclusion complex formed. Preferably the composition contains between 5 and 95% alpha-glucan and between 95 and 5% guest molecule. It may be preferable, if the guest molecule is particularly potent, to use a larger proportion of alpha-glucan.

A tenth aspect of the invention provides use of inclusion complexes of alpha-glucans as excipients. The invention also provides use of inclusion complexes of the invention in the preparation of a composition for use in therapy.

The invention will now be described with reference to the following examples. Variations on these falling within the scope of the claims will be apparent to a person skilled in the art.

EXAMPLES

EXAMPLE 1 - USE OF MECHANICALLY DAMAGED STARCH AS A TABLET EXCIPIENT

Preparation of Damaged Starch

Damaged starch was produced by ball-milling maize (Fisons S/7880/60), potato (BDH 30262) or laboratory extracted starches for various times in a Pascall ball-mill. The amount of damage was determined according to Karkalas *et al.* (J Karkalas, RF Tester and WR Morrison, J Cereal Science, 16 (1992) 237 - 2551) as shown in Table 1.

Table 1. Damage in ball-milled starches

Sample (% damage)	Damage, % wb[1]	Damage, % db[2]
Native maize (M0)	0.6	0.7
Native potato (P0)	0.6	0.7
Ball-milled maize (BMM30)	30.3	34.0
Ball-milled potato (BMP30)	32.1	37.8
Ball-milled maize (BMM80)	81.8	91.9
Ball-milled potato (BMP80)	86.2	100.0

[1] wet basis, [2] dry basis

Different amounts of damage were introduced into a range of cereal and tuber starches and their functionality as tablet excipients was investigated.

Formulation 1

The general tablet formulation is presented in Table 2.

Table 2. General formulation for production of 350 mg tablets - Formulation 1

Component	Amount, %
Aspirin	94.74
Starch	4.21
Magnesium Stearate	1.05

The starch component of the tablets contained either native, circa 30% damaged or circa 80% damaged maize or potato starch (Table 3), with a tablet weight of 350 mg.

Table 3. Amount of native and damaged starch in 350 mg tablets - Formulation 1

% Damage	Starch	Amount of starch in tablets, mg (db)[1,2]	
		Native	Damaged
0	Maize	13.02	0.09
30	Maize	8.65	4.46
80	Maize	1.06	12.05
0	Potato	12.43	0.09
30	Potato	7.79	4.73
80	Potato	0	12.52

[1]dry basis

[2]Where total starch (dry mass) content of 350 mg tablets is 13.11 mg for maize and 12.52 mg for potato starch

Formulation 2

Tablets were also produced using a formulation including a carboxymethylcellulose (Avicel) (Table 4).

Table 4. General formulation for production of 350 mg tablets - Formulation 2

Component	Amount, %
Aspirin	0-94.74
Starch	4.21
Avicel(TM)	0-94.74
Magnesium Stearate	1.05

Formation of Tablets

Tablets were produced using a Manesty hand tableting machine.

Tablet Hardness

Hardness was measured using Scheleuniger hardness testing apparatus where hardness was measured in Kp.

Disintegration Testing

Standard Method

Disintegration was also determined according to standard BP disintegration methodology using Erweka apparatus.

Laboratory Water Bath Method

Disintegration rates for the tablets were determined by shaking individual tablets (and commercial paracetamol tablets as controls) in 5 ml water, 2M HCl or 1 mg ml^{-1} α-amylase solution (Karkalas *et al.*, 1992), within sealed 10 ml screw-cap tubes arranged horizontally in a shaking water bath (60 cycles per minute) at 37°C.

Results

Formulation 1

Standard Methods

Using the standard methods for determining hardness and disintegration the following data (Table 5) was obtained.

Table 5. Hardness and disintegration time (standard BP methods) for tablets produced containing native, circa 30 or circa 80% damage

% Damage	Starch	Hardness, Kp	Disintegration Time, (min)
0	Maize	7.7 ± 1.1	14.2 ±1.8
30	Maize	nd	nd
80	Maize	7.4 ± 0.3	>15
0	Potato	7.5 ± 0.8	1.8 ± 0.7
30	Potato	8.4 ± 0.4	>15
80	Potato	7.8 ± 0.2	>15

Disintegration rates for the tablets made according to formulation 1 using the laboratory water bath method (shaken in water, 2M HCl or α-amylase solution) are presented in Table 6.

Table 6. Disintegration time for tablets containing native or damaged starch using the laboratory water bath method, with water or α-amylase solution

% damage	Starch	Disintegration Time (min)		
		Water	2M HCl	α-amylase
PAR*	Maize & Others	1	1	1
M0	Maize	6	7	6
BMM80	Maize	17	>120	17
P0	Potato	1	1	1
BMP80	Potato	>180	>180	>180

* Commercial Paracetamol tablets (500 mg) containing paracetamol, maize starch, potassium sorbate, purified talc, stearic acid, polyvidone, soluble starch, hydroxypropylmethylcellulose and triacetin.

Contrary to what might be expected, the damaged starches (and especially the extensively ball-milled potato starch which was the most physically modified) increased disintegration times in water, HCl and α-amylase. There was little effect on hardness. One possible explanation for this result being that although amorphous starches readily hydrate and swell in water and are more easily hydrolysed by HCl and α-amylase when dispersed as starch powders, when the relatively hydrophobic tablet formulations were shaken in water, acid or enzyme a surface gel layer formed from the damaged starch which resisted water, acid or enzyme penetration because of its association with the aspirin. The acid maintains the aspirin in its hydrogen form which is much less soluble than for example the sodium salt (which is present in soluble apirin formulations). However, it is recognised that starch does interact with aspirin (VC Okore, STP Pharma Sciences 4(5) (1994) 373-376) and that this interaction may be greater with damaged starch and hence control its release from tablets.

It is proposed that extensively ball-milled (amorphous) starch can be used in certain tablet formulations as a slow or controlled release regulator of vitamins or drugs.

Formulation 2

When a microcrystalline cellulase (Avicel™) was incorporated into the tablet formulation, the following data (Table 7) was obtained:-

Table 7. Hardness and disintegration time (standard BP methods) for tablets produced containing Avicel and native or cica 30% damaged starch

% damage	Starch	Hardness, Kp	Disintegration Time, (min)
0	Maize	Off Scale	3
30	Maize	Off Scale	>>15
0	Maize	Off Scale	3
30	Maize	Off Scale	>>15

Carboxymethylcellulose is used as a tablet excipient in a number of products because of its rapidly swelling and hydrating properties. When used in these formulations with starch, however, the gel that is formed from the damaged starch restricts hydration of the tablets as discussed above. When a hydration 'wick' like silica was incorporated, however, initial results indicate that hydration channels are formed which facilitate the disintegration of tablets containing damaged starch because the damaged fraction hydrates and swells without forming an impermeable barrier to the hydration of the tablets. Using this formulation, the damaged starch has the capacity to act as a rapid rather than slow release disintegrant.

Pre-swelling of Starch

Although damaged starch may be incorporated into tablet formulations as a simply mechanically modified excipient, it may also be pre-swollen in th presence of a chemical moiety in solution which is then drawn into the matrix of the swollen granules. Upon

drying a fairly rigid gel is formed if the starch is extensively mechanically damaged, although less swelling and gel is formed if the initilal amount of damage is low.

Procedure

Samples (500 mg) of severely damaged maize and potato starch (80% damage) were weighed into 10 ml screw cap tubes. To the samples, different volumes (0.1-1 ml) of ascorbic acid solution (100 mg ml^{-1}) were added by pipette and the tubes were mixed (at room temperature). The starches spontaneously swelled; the more ascorbate solution that was added the greater the swelling. The starches were then either freeze or air dried.

Different water soluble compounds (e.g. drugs) may be incorporated into the swollen gel matrix using this procedure and by using different water-miscible solvent carrier systems. The starches may be heated although this is not necessary if the starches are extensivley modified since they spontaneously swell at room temperature. It is possible to dehydrate the swollen hydrated gels by adding excess ethanol, mixing, centrifuging (e.g. 1,500 x g for 5 minutes) and discarding the ethanol. If swelling is, however, excessive the swollen material is difficult to manipulate. At lower levels it is easier. Fat soluble chemical moieties (e.g. drugs) can then be added to the swollen starches in organic solvents (e.g. ethanol) after which the solvent can be evaporated.

The swollen damaged starches containing a guest moiety as described above may be incorporated into drugs formulations (e.g. tablets) after grinding to a powder. Grinding in a pestle and mortar is a very efficient way of doing this.

Additional Experiments

Studies involving disintegration tests on tablets containing aspirin or ibuprofen, Avicel™ (microcrystalline cellulose), starch containing different levels of damage and stearic acid indicate that damage has a reproducibly different effect on tablet disintegration depending on the level of damage and tablet formulation. Depending on the formulation there can be enhancement of disintegration because the granules readily hydrate and swell. In other formulations the surface gel layer prevents diffusion of water into the tablets and resists disintegration.

It is recognised that regardless of damage, the amount of starch and the starch to Avicel™ ratio in tablets has a marked effect on tablet disintegration and dissolution characteristics of aspirin (Torrado-Santiago *et al.*, 1995). Damage is superimposed on the starch content and interactions between the damaged starch and any other components of the tablets cannot be ignored.

It is proposed that physically damaged starch may be used as a novel tablet excipient in place of native, pre-gelatinised or chemically/enzymatically modified starches in particular formulations. They could be ingested or administered to the GI tract via the rectum, formulated for inhalation or parenteral administration.

EXAMPLE 2 - USE OF SEMI-GELATINISED STARCHES AS A DRUG/CHEMICAL DELIVERY SYSTEM

Testing of Approach

Any starches may be used. The following serves to illustrate how water and fat soluble materials may be surface coated on to/impregnated in to starches.

Method I Water Soluble Glutamine

A *Preparation of Gelentraped Compositions - Water Soluble Glutamine*

Samples (1 g) of potato starch (e.g. BDH 30262) were weighed into 20 ml screw-cap tubes. In addition 100 mg glutamine (e.g. Sigma G-9003) was weighed into the tubes and the contents were manually blended (e.g. stirring rod). To this blend 1 ml distilled water was added by pipette and the potential guest molecule was dissolved (as far as possible). Other potential water soluble guest molecules can be used and alternatively the guest molecule can be pre-dissolved in the water (1 ml) rather than during mixing of the dispersed starch. The tubes were sealed and heated at 55°C (e.g. water bath or oven) or other appropriate temperature within the gelatinisation onset and conclusion temperatures by DSC for an appropriate time (e.g. 30 minutes). This permits only partial gelatinisation of the starch but allows permeation by potential guest molecules. The starches were cooled to room temperature and then dried (air or preferably freeze dried) and air equilibrated to about 11% moisture.

B *Testing Extent of Association*

Samples of starch (50 mg, equivalent to 4.55 mg glutamine) were weighed into 10 ml screw cap tubes. To these tubes 5 ml distilled water was added by pipette and the tubes were sealed. Extraction in water at different times (0 to 120 minutes) was achieved by placing the tubes horizontally in a shaking water bath (approximately 60 cycles per minute) at 40°C. The tubes were then cooled to room temperature and centrifuged (1,500 x g for 5 minutes). An aliquot (1 ml) of the supernatant was removed by pipette and the glutamine content was determined (after appropriate dilution) according to the associated unpublished method of Karkalas, calibrated using a standard glutamine solution.

Briefly this method is performed as follows.

DETERMINATION OF ALPHA-AMINO NITROGEN

Reagents

Ninhydrin reagent: Weigh individually 0.5 g of ninhydrin, 0.3 g of fructose, 10 g Na_2HPO_4 (anhydrous) and 6 g KH_2PO_4, dissolve in water and make up to 100 ml in a volumetric flask. Store in a brown bottle at 4°C, max. 1 week.

Ethanolic potassium iodate: Measure 40 ml ethanol in a graduated cylinder and add water to the 100 ml mark. Transfer into a beaker containing 1 g of KIO_3 and stir with a magnetic stirrer for 2 hours to saturate the solution. Filter the solution and store the filtrate in a stoppered flask.

Stock solution of leucine: Dissolve 93.5 mg of leucine in 100 ml of water in a volumetric flask (1 ml ≡ 0.1 mg α-amino N).

Standard solutions of leucine: Transfer 1, 2, 3, 4 and 5 ml of stock solution respectively into 100 ml volumetric flasks and make up to volume with water (1-5 µg/ml).

Procedure

Into stoppered tubes (10 ml capacity) transfer 2 ml of standard solution, or sample, and add 1 ml of ninhydrin reagent. Vortex mix. As a reference use 2 ml of water and 1 ml reagent. Protect from intense light.

Place in a boiling water bath for 15 minutes, then cool in running tap water for 5 minutes. To all tubes add 5 ml of ethanolic potassium iodate, mix contents by repeated inversions of the tubes, and read the absorbance at 570 nm within 30 minutes.

Plot concentration of α-amino nitrogen versus absorbance, calculate the regression equation for the straight line and the correlation coefficient. Calculate the α-amino nitrogen content of the sample.

N.B. Ammonia interferes with this determination and should be absent. If ammonia is present, it should be determined separately by e.g. distillation or by the colorimetric method enclosed herewith.

In some situations it is difficult to dissolve (apparently) water soluble materials. Phenylalanine is a good example but other water soluble molecules can be treated in the same way (subject to solubility). The difficulty here is making a concentrated starch;water slurry (which restricts starch gelatinisation) and at the same time facilitating solubilisation and impregnation by the amino acid. To overcome this problem phenylalaline (Sigma P-8324, 500 mg) was weighed into a 100 ml beaker and 10 ml water was added by pipettte. The beaker was suspended in a boiling water bath and the contents were mixed to dissolve the amino acid. The beaker was then transferred to a 55°C water bath. After temperature equilibration (c. 5 minutes), 10 g of potato starch was *rapidly* added to the phenylalanine solution and thoroughly mixed to maximise amino acid association with the starch. After 30 minutes, the beaker was removed from the heat and the contents were either air or freeze dried.

Different time and temperature protocols can be used. However, the important thing to do when adopting this approach is to rapidly add the starch and immediately mix to prevent regions of high water to starch being present in the beaker. These can cause more extensive gelatinisation and swelling than is desirable. Freeze drying is preferable to air drying since granulation is reduced and a more free flowing powder is obtained.

Testing Extent of Association - when the amount of gelatinised starch (from DSC endotherm, see below) is less than 35% Glutamine

Samples of starch (50 mg, equivalent to 4.55 mg glutamine) were weighed into 10 ml screw cap tubes. To these tubes 5 ml distilled water or α-amylase (Sigma A-0273,

from *Aspergillus oryzae*) solution (1 mg ml^{-1}, according to Karkalas *et al.*, 1992) was added by pipette and the tubes were sealed. Extraction in water at different times (0 to 120 minutes) was achieved by placing the tubes horizontally in a shaking water bath (approximately 60 cycles per minute) at 40°C. The tubes were then cooled to room temperature and centrifuged (1,500 x g for 5 minutes). An aliquot (1 ml) of the supernatant was removed by pipette and the glutamine content was determined (after appropriate dilution) according to the associated unpublished method of Karkalas, calibrated using a standard glutamine solution.

Testing Extent of Association - when the amount of gelatinised starch (from DSC endothem, see below) is more than 50% Phenylalanine

The same basic procedure was adopted as described for glutamine (above) although samples were extracted, in water, 2M HCl or α-amylase at 40°C for 2 hours.

Results 1 Water Soluble Extraction - Glutamine

Table 8. Extraction of glutamine from freeze dried glutamine impregnated potato starch (50 mg) heated in 5 ml water at 40°C

Sample Number	Extraction Time (min)	Amount of Glutamine Extracted* (mg)	Amount of Glutamine Extracted*, %
G0	0	0.33 ± 0.01	7.25
G15	15	0.34 ± 0.01	7.47
G30	30	0.39 ± 0.02	8.57
G60	60	0.36 ± 0.01	7.91
G120	120	0.34 ± 0.00	7.47

* Where total extractable is 4.55 mg

These data (Table 8) indicate that under the extraction conditions used, only a small proportion of the glutamine (9%) was extracted and this was essentially independent of time.

The amount of amorphous material (or 'damage') in this modified (pre-swollen) starch was compared to the native starch using the method of Karkalas *et al.* (1992) which incorporates hydrolysis of amorphous starch by 1 mg ml^{-1} α-amylase (*Aspergillus oryzae*) when heated for 15 minutes at 30°C. The method was, however, adapted (to 'exaggerate' body temperature) where hydrolysis was performed at 40°C rather than at 30°C and hence 'apparent damage' was determined (Table 9).

Table 9. Apparent damage levels for freeze dried glutamine impregnated starch

Starch	Apparent Damage, %
Native Potato	0.7
Glutamine Bound	11.60

The increase in 'damage' identified when comparing native to glutamine impregnated starch is caused by the temperature driven association of the starch with the glutamine, and reflects the partially gelatinised nature of the starch. It is evident, however, that only a small level of modification occurs (<12%) and the starch retains most of its semi-crystalline characteristics.

Under the conditions of hydrolysis used for the modified damaged starch assay, the freely extractable (solubilised) glutamine content was measured (as previously described) and compared to the amount solubilised without enzyme present (Table 10).

Table 10. Glutamine extractable from glutamine impregnated starch with or without enzyme digest of starch (15 minutes incubation at 40°C

Starch Treatment	Glutamine Extracted*, %
Water Extraction	7.47
After α-amylase Hydrolysis of Amorphous Starch at 40°C	19.16

*Where total extractable is 4.55 mg

There was a substantial increase in glutamine released upon hydrolysis of the amorphous material.

The gelatinisation parameters by DSC were determined to characterise how much modification to the native starch had occurred during processing (Table 11).

Table 11 Gelatinisation parameters by DSC for native and glutamine impregnated potato starch (freeze and air dried)

Starch	Gelatinisation Temperature, °C			Enthalpy
	T_o	T_p	T_c	(J/g)
Native	55.0	64.7	76.0	15.25
Glutamine Impregnated (Freeze Dried)	56.0	62.3	75.0	10.09
Glutamine Impregnated (Air Dried)	63.0	67.3	77.0	12.30

The increase in T_o and reduction in enthalpy confirms that only partial gelatinisation of the starch had occurred during the processing designed to incorporate the glutamine.

Results Water Soluble Extraction - Phenylalanine

The amount of phenylalanine extracted from starch in water, HCl or α-amylase solution was dependent on the conditions used to associate the amino acid with the starch during partial gelatinisation (starch to water ratio, temperture, time and phenylalanine concentration). Using a starch preparation where the enthalpy had been reduced substantially as a consequence of gelatinisation during processing (7.28 J/g compared to 15.25 J/g for the native starch; representing on this basis 52% gelatinised material) the following extractions (Table 2) were achieved.

Table 12. Extraction of phenylalanine from extensively gelatinised starch (250 mg) in water, 2M HCl or α-amylase s lution at 40°C (2 hour extraction)

Extractant	Amount Extracted* mg	Amount Extracted* %
Water	10.79 ± 0.43	90.7
2M HCl	11.25 ± 0.02	94.5
α-amylase	11.04 ± 0.48	92.7

*Where total extractable is 11.90 mg

Where there is quite extensive gelatinisation as in this example, it is relatively easy to extract the guest molecule. These materials would be suitable for more rapid release of guest molecule in tablet formulations although solubilisation could be controlled by the use of damaged starch (Part I) in the tablet formulations.

C *Preparation of Gel Entrapped Compositions - Water Soluble Ascorbate*

The exercises (above) were repeated using ascorbic acid (1 g potato starch plus 100 mg ascorbic acid, BDH 10303) where the ascorbic acid extracted was quantified (after appropriate dilution) by iodophenol titration (Kirk and Sawyer, (1991) Pearson's Composition and Analysis of Foods, 9th Edition, pp 264, Longman Scientific and Technical) and the BCL diagnostic colorimetric method (Catalogue Number 409 677). The heating protocol used to associate the ascorbate with the starch (heating at 55°C for 30 minutes to impregnate starch and 40°C for 120 minutes during leaching experiment) was found to have no destructive effect on the vitamin using standard ascorbic acid solutions (0.1 and 1%) and the quantification methods described above (Table 13).

Table 13. Effect of temperature on the asc rbic acid concentration of a solution (1%) f the vitamin

Condition	Ascorbic Acid Content, %
Standard Solution	1.00
Heated 55°C (30 minutes)	1.00
Heated 40°C (120 minutes)	1.00

D *Testing Extents of Association - Water Soluble Ascorbate*

Extraction was quantified with respect to incubation time as shown in Table 14.

Table 14. Extraction of ascorbic acid from freeze dried ascorbate impregnated potato starch (50 mg) heated in 5 ml water at 40°C

Sample Number	Extraction Time	Amount of Ascorbate Extracted* (mg)	Amount of Ascorbate Extracted*, %
A0	0	0.5 ± 0.1	11
A15	15	0.4 ± 0.2	9
A30	30	0.2 ± 0.2	4
A60	60	0.4 ± 0.1	9
A120	120	0.3 ± 0.2	7

*Where total extractable is 4.55 mg

Apparent damage was quantified (Table 15).

Table 15. Apparent damage levels for freeze dried ascorbate impregnated starch

Starch	Apparent Damage, %
Native Potato	0.7
Ascorbate Bound	15.70

Ascorbate extraction post amorphous starch hydrolysis with α-amylase is shown in Table 16.

Table 16. Ascorbate extracted from ascorbate impregnated starch with or without enzyme digest of starch (15 minute incubation at 40°C)

Starch Treatment	Ascorbate Extracted, %
Water Extraction	7
After α-amylase Hydrolysis of Amorphous Starch	5

The limited amount of α-amylase hydrolysis did not increase the amount of ascorbic acid which was able to be extracted from the starch and indicates that a stronger association occurred with this vitamin than glutamine.

Gelatinisation characteristics for ascorbate bound starches using DSC are shown in Table 17.

Table 17. Gelatinisation parameters by DSC for native and ascorbate impregnated potato starch (freeze and air dried)

	Gelatinisation Temperature, °C			Enthalpy
Starch	T_o	T_p	T_c	(J/g)
Native	55.0	64.7	76.0	15.25
Ascorbate Bound (Freeze Dried)	58.0	65.1	77.0	8.68

Hence quite substantial modification to the starch had occurred during processing as indicated by the increase in T_o and decrease in enthalpy. Note that under these conditions of association and extent of gelatinisation (as indicated by the enthalpy) there is a much stronger association of the vitamin with the starch (presumably because of its greater ease of penetration of starch granule when swollen in the presence of the vitamin and greater level of native crystallinity) than was found for phenylalanine (as described previously).

E *Preparation of Gel Entrapped Compositions - Lipid Soluble Molecules*

Fatty Acids

When lipid soluble molecules are used as guest molecules, a slightly different approach is necessary. The starch has to be pre-swollen in water (since starch cannot be swollen in organic solvents) and then the lipophilic molecule is added.

Potato starch was pre-swollen in water as discussed above (1 g heated in 1 ml water at 55°C for 30 minutes). Lipophilic molecules were added to the starch in an appropriate water miscible solvent, (alcohols and acetone are examples) where ethanol was preferred. Examples of this approach are illustrated as follows:-

A 150 mg sample of palmitic acid (Sigma P-0500) was dissolved in 4 ml ethanol. A 1 ml aliquot (37.5 mg) was added by pipette to 1 g unswollen (native) potato starch in a 20 ml screw cap tube. Similarly 1 ml was added to 1 g of the pre-swollen starch. The solvent (and hence the fatty acid) were blended into the starch using a fine glass rod and the tubes were left at room temperature for 30 minutes. The ethanol was evaporated in a fume cupboard using a stream of nitrogen and the residual water from the pre-swollen starch was removed by air drying.

Water saturated butanol (WSB) may be used to extract flour or starch surface lipids (WR Morrison, DL Mann, W Soon and AM Coventry (1975) Journal of the Science of Food and Agriculture, 26, 507 - 521) and this solvent was used to identify if the unswollen starch more easily liberated lipid (since the lipid did not impregnate the starch) than the pre-swollen starch.

Quadruplet samples (100 mg) of unswollen (surface coated) or pre-swollen (impregnated) starch were weighed into 10 ml screw cap tubes. To each tube 5 ml WSB was added by pipette and the tubes were placed in an empty shaking (60 cycles per minute) water bath at room temperature for 15 minutes. The tubes were then centrifuged (1,500 x g) for 5 minutes. From each tube 1 ml was removed by pipette and transferred to clean 25 ml volumetric flasks and the flasks were made up to volume with methanol before mixing. From each flask 5 ml was removed by pipette and transferred to clean 10 ml screw cap tubes containing 100 µl (100 µg) of margaric acid (Sigma H-3500) as a calibration standard. The solvent from each tube was then evaporated under nitrogen.

To each tube 2 ml boron trifluoride methanol complex (Fisons) was added by pipette. The sealed tubes were then boiled for 15 minutes (boiling water bath) and then cooled to room temperature. Next, to each tube 1 ml distilled water and 2 ml pentane were added by pipette. The tubes were shaken to dissolve the fatty acid methyl esters in the pentane. The pentane was removed by pipette and transferred into clean tapered 10 ml screw cap tubes and then evaporated under nitrogen. Finally, to each tube 100 µl diethyl ether was added by pipette and the tubes were vortex mixed. Aliquots (10 µl) were injected into a Perkin Elmer Autosystem Gas Liquid Chromatograph operating at 185°C with helium as a carrier gas and separating on a Supelco SP-2380 fused silica capillary column with FID detection. Quantification was based on the quantity of C17 calibration standard.

Results - Fatty Acids

There was significant difference between the amount of palmitic acid extractable from the surface coated compared to the impregnated starch (Table 18).

Table 18. Amount of added palmitic acid extractable from surface coated and impregnated potato starch

Condition of Starch	Total Amount Extractable from 100 mg	Amount Extracted from 100 mg Starch, mg	Amount Extracted from 100 mg Starch, %
Surface Coated	3.61	3.29 ± 0.10	91.14
Impregnated	3.61	2.66 ± 0.25	73.68

This indicates that the fatty acid molecules have penetrated and become more tightly associated with the pre-swollen starches than the unswollen control samples.

F *Method - Aspirin Formulations*

Starches

Samples (1 g) of potato starch were weighed into 20 ml screw cap tubes. To the tubes, 1 ml water was added by pipette and after sealing they were heated at 55°C for 30 minutes. After cooling to room temperature, 9 ml absolute alcohol was added by pipette and the tubes were re-sealed. Next the tubes were shaken for 5 minutes at room temperature and then allowed to equilibrate for 15 minutes. Finally, the tubes were centrifuged (1,500 x g for 5 minutes) and the ethanol was decanted and discarded.

To samples (1 g) of unswollen or swollen (as described above) potato starch, 1 ml of ethanol containing 125 mg^{-1} aspirin (Sigma A-5376) was added by pipette. The solution was thoroughly mixed with the starch and a thin glass rod and then allowed to equilibrate for 30 minutes. After this time, warm air was circulated through the tubes and the ethanol was evaporated. The starches were then placed on a small glass dish and allowed to equilibrate to 11% moisture.

Method - Aspirin Extractability

Triplicate samples (50 mg) of unswollen (surface coated) and swollen (impregnated) aspirin treated starches were weighed into 10 ml screw cap tubes. To each tube 10 ml distilled water or α-amylase solution (J Karkalas, RF Tester and WR Morrison, J Cereal Science, 16 (1992) 237 - 2551) was added by pipette and the sealed tubes were placed in a shaking water bath (c. 60 cycles per minute) at 37°C for 30 minutes. After extraction the tubes were centrifuged (1,500 x g for 5 minutes) and the absorbance of a 1 in 50 dilution of the supernatant was measured at 226 nm (against appropriate blanks containing no starch) and converted to amount of aspirin leached into 10 mls by reference to a standard curve.

Results - Aspirin Extractability

The amount of aspirin extracted from the surface coated and impregnated starches in water is presented in Table 19.

Table 19. Extractability of aspirin from surface coated and impregnated potato starch (50 mg) extracted for 30 minutes in 10 ml water at 37°C

	Amount Extracted*, mg	Amount Extracted*, %
Surface Coated	5.3 ± 0.3	95.4
Impregnated	3.9 ± 0.2	70.2

*Where starches are prepared with 5.56 mg aspirin per 50 mg starch.

When the exercise was repeated using impregnated starch extracted in α-amylase there was a small increase in the amount of aspirin extracted (Table 20).

Table 20. Extractability f aspirin from impregnated potato starch (50 mg) extracted for 30 minutes in 10 ml α-amylase at 37°C

	Amount Extracted*, mg	Amount Extracted*, %
Impregnated	4.2 ± 0.2	75.5

*Where starches are prepared with 5.56 mg aspirin per 50 mg starch.

These results show that pre-swollen starch can be used to delay aspirin solubilisation from a starch matrix.

G. *Association with Retrograded Amylose*

Retrograded amylose was prepared as described in Part III. Samples (100 mg) of the starches which had been surface coated or impregnated with aspirin were mixed with retrograded amylose gel (containing about 150 mg α-glucan and about 800 µl water) and then either air or freeze dried. Initial experiments indicated that the dry surface coating of retrograded amylose made it extremely difficult to solubilise the aspirin and this enhancement of the system might be suitable for slow release of drugs in the colon of man.

EXAMPLE 3

Preparation of Inclusion Complexes of α-Glucans

Potato amylose (Sigma A-9262) was dissolved in dimethyl sulphoxide (DMSO) to give an estimated concentration of 30 mg ml^{-1}. Samples (5 ml aliquots) were pipetted into 100 ml screw top flasks. To these flasks, 40 ml hot (85°C) distilled water was added by pipette followed immediately by a 5 ml aliquot of potential guest molecule dissolved in methanol or other solvent. These included *trans*-β-carotene (Sigma C-9750), aspirin (acetylsalicylic acid, Sigma A-5376), folic acid (Sigma F-7876) ibuprofen (α-methyl-4-[isobutyl]phenylacetic acid (Sigma I-4883) and retinol acetate (Sigma R-4632). All potential guest molecules were dissolved in methanol except β-carotene which was dissolved in chloroform (although any appropriate solvents can be used), giving a concentration range of 1 to 5 mg ml^{-1}. A 3 mg ml^{1} mono-myristin (Sigma M-1890) standard was also used which is an established guest molecule in amylose-lipid complexes

(J Karkalas, S Ma, WR Morrison and RA Pethrick, Carbohydrate Research, 268, (1995) 233 - 247) plus methanol alone (solvent with no solubilised guest molecule). The flasks were immediately sealed and shaken. As mentioned above, many other drugs, chemicals (e.g. dyes) and related substances might be used in place of these compounds, and in different concentrations although they serve as an example of potential drug delivery systems.

Other solubilisation systems were also used to form the associations and complexes including amylose directly solubilised in water, alkaline solutions and amylose solubilised and re-generated from amylose-butanol complexes. It should be recognised that these associations and inclusion complexes may be produced from a number of starting points and solvent systems at different temperatures.

The flasks were stored at different times and at different temperatures to facilitate complex formation. One example was for 30 minutes at 5°C another was at 20°C for 18 hours. After storage the liquor was shaken and poured into 70 ml glass centrifuge tubes which were centrifuged at 3,000 rpm (1,700 x g) for 15 minutes at 7°C. Aliquots (0.5 ml) of the supernatant were transferred to clean 10 ml screw cap tubes by pipette and the α-glucan (amylose) content was determined according to Karkalas (1985). The residual liquor was decanted and the precipitate was freeze dried. After (and before) freeze drying the complexes were characterised using a Mettler Differential Scanning Calorimeter (DSC 10A Professor and DSC 30 Low Temperature Cell) where samples (5 mg) of dried material were heated with 15 µl water (or 20 mg un-dried) in 40 µl aluminium pans from 30 to 130°C at 10°C min^{-1}.

Diffusion of ibuprofen from the freeze dried precipitates formed using 1 to 5 mg ml^{-1} ibuprofen was assessed in water and α-amylase solutions. Accurately weighed samples (10-20 mg) of the precipitates were weighed into 10 ml screw cap tubes and either 10 ml distilled water or 1 mg ml^{-1} α-amylase solution (J Karkalas, RF Tester and WR Morrison, J Cereal Science, 16 (1992) 237 - 2551) was added by pipette. The sealed tubes were placed in a shaking water bath at 37°C for 30 minutes (at 70 cycles per minute). After extraction the tubes were centrifuged at 3000 rpm (1,500 x g) for five minutes and the absorbance of the supernatant at 220 nm was recorded and converted to amount of ibuprofen extracted by reference to a standard ibuprofen solution.

Results

Analytical

When samples were rapidly cooled, precipitate was visible in all samples including amylose alone (Table 21).

Table 21. Experiment to identify if associations/complexes could be produced with amylose when rapidly cooled and precipitated at 5°C for 30 minutes

Condition	Amylose Content per Supernatant of 50 ml flask, mg*	Proportion of Initial Amylose Content in Complexed or Retrograded, %
Standard Amylose Solution[1]	147.5	0
Amylose[2]	47.3	68.0
Mono-Myristin (3 mg ml^{-1})	4.0	97.3
Aspirin (3 mg ml^{-1})	30.9	79.0
Ibuprofen (3 mg ml^{-1})	9.5	93.6

*According to Karkalas (1985)

[1] Represents the standard amylose solution before use in complexing system

[2] Represents amylose content in 50 ml flask where no guest was incorporated

In all cases, the addition of a potential guest molecule increased the amount of amylose precipitated from solution (compared to amylose with no addition). The precipitate was quite different in the mono-myristin and ibuprofen systems compared to the amylose (alone) and aspirin. The former were 'gritty' and definite precipitates whilst the latter were floccular. This evidence indicates that the former contained complexed material as opposed to the latter which were more extensively retrograded amylose with a lower content of any true complexes. This was confirmed by DSC on the wet ibuprofen precipitate which had a dissociation endotherm with a peak temperature of 114.7°C and an enthalpy of approximately 7.2 J/g. If this material had been simply retrograded amylose it would have had a transition temperature of about 140-150°C and hence it appeared that true inclusion complex was made.

When the experiment was repeated using a complexing temperature of 20°C for 18 hours the following results were obtained (Table 22).

Table 22. Experiment to identify if associations/complexes could be produced with amylose when slowly precipitated at 20°C

Condition	Amylose Content per Supernatant of 50 ml flask, mg*	Proportion of Initial Amylose Content in Complexed or Retrograded, %
Standard Amylose Solution[1]	147.5	0
Aspirin (3 mg ml^{-1})	20.2	86.3
β-Carotene (3 mg ml^{-1})	4.7	96.8
Folic Acid (3 mg ml^{-1})	27.9	81.1
Ibuprofen (1 mg ml^{-1})	18.1	87.8
Ibuprofen (2 mg ml^{-1})	17.1	88.4
Ibuprofen (3 mg ml^{-1})	9.2	93.8
Ibuprofen (4 mg ml^{-1})	7.5	94.9
Ibuprofen (5 mg ml^{-1})	6.0	95.9
Retinol acetate (3 mg ml-1)	18.2	87.7

*According to Karkalas (1985)

[1] Represents the standard amylose solution before use in complexing system

The results show that there is a concentration dependence for association/complex formation (Ibuprofen) and that there is variation in the amount of material (association/complex) formed when different associating/guest molecules are used. Similar results to those presented for folic acid were obtained when the anti-cancer analogue of folic acid (amethopterin or methotrexate) was used.

The β-carotene and folic acid precipitates are orange and yellow respectively whilst the supernatants were clear indicating that regardless of whether or not true inclusion complexes are formed, associations were formed as a consequence of amylose retrogradation and precipitation from solution. Upon freeze drying, the gels became very rigid and hard.

Differential Scanning Calorimetry

Data for DSC are presented in Table 23. Dried amylose gels had no discernible endotherms although at 130°C the baseline began to shift towards what appeared to be the onset of dissociation of retrograded amylose, which it is anticipated would have a peak dissociation temperature of about 145°C (Karkalas, 1985; Karkalas and Raphaelides, 1986; Karkalas *et al.*, 1995: Raphaelides and Karkalas, 1988). For the β-carotene, folic acid and retinol acetate no readily detectable endotherms were obvious below this temperature indicating that any of these molecules were trapped within the matrix of retrograded amylose and were not necessarily true inclusion complexes.

Table 23. Characterisation of amylose-vitamin and amylose drug precipitates by DSC

Association/ Complex	True Melting Point (°C)	Transition Temperature (°C)			Enthalpy (J/g)
		T_o	T_p	T_c	
Amylose	No distinct endotherm until circa 130°C				
Aspirin (3 mg ml^{-1})	138-140	122	126.7	135	0.6
β-Carotene (3 mg ml^{-1})	178-179	No distinct endotherm until circa 130°C			
Folic Acid (3 mg ml^{-1})	No distinct endotherm until circa 130°C				
Ibuprofen (1 mg ml^{-1})	51-53	115	123.1	128	1.1
Ibuprofen (2 mg ml^{-1})	51-53	113	121.1	130	6.8
Ibuprofen (3 mg ml^{-1})	51-53	113	119.7	132	13.5
Ibuprofen (4 mg ml^{-1})	51-53	115	119.7	140	11.7
Ibuprofen (5 mg ml^{-1})	51-53	111	122.4	141	16.3
Retinol Acetate (3 mg ml^{-1})	c. 58	No distinct endotherm until circa 130°C			

A very small peak (peak temperature 126.7°C) was found for the aspirin sample indicating that some complexing had occurred. For the ibuprofen, the situation was much clearer where distinct endotherms were detected with onset, peak and conclusion temperatures of between 111-115, 120-123 and 128-141 °C respectively. As the amount of guest molecule was increased to about 10 parts amylose to 1 part ibuprofen (i.e. 3 mg ml^{-1}), the enthalpy tended to stop increasing (13.5-16.3 J/g) in a concentration dependent manner indicating that in common with fatty acids (Karkalas *et al.*, 1995) an optimum amylose to guest molecule ratio had been obtained for saturation of the amylose helices.

Additional proof for the formation of amylose-ibuprofen interactions (associations or complexes) was obtained by measuring the absorbance of 1 in 500 dilutions (water) of the samples at the λ_{max} (220 nm) of the 3 mg ml^{-1} ibuprofen solutions or supernatants immediately post addition of ibuprofen, after refrigeration for 30 minutes or after 18 hours storage at 20°C. These data are presented in Table 24.

Table 24. Reduction in absorbance (220 nm) of amylose-ibuprofen (3 mg ml^{-1}) mixtures (diluted 1 in 500) as a function of temperature and time

Condition	Absorbance (220 nm)
Initial Solution	0.596 ± 0.005
Post Refrigeration (30 minutes)	0.580 ± 0.002
Post 20°C Storage (18 hours)	0.567 ± 0.001

The decrease in absorbance is against a background in absorbance of the DMSO but clearly shows a downward trend which is attributed to reduction in ibuprofen content of the flasks post association or complexing. If there had been no association or complexing the absorbance would have remained unchanged from the initial solution absorbance (0.596 at 220 nm).

Ibuprofen Extraction in Water and α-amylase

After extraction in water or α-amylase (37°C for 30 minutes), there was very little visible difference in the amount of precipitate in the tubes and hence there appeared to be little solubilisation. The amount of ibuprofen extracted under the conditions used is

presented in Table 25 and shows that only a small amount of aspirin was released in either water or the enzyme solution.

Table 25. Extraction of ibuprofen from freeze dried precipitates (complexes)

Original Ibuprofen Precipitation System	Ibuprofen Extracted (mg per 100 mg Precipitate)	
	Water	α-amylase
1 mg ml^{-1}	1.7	2.4
2 mg ml^{-1}	2.1	2.5
3 mg ml^{-1}	1.9	nd
4 mg ml^{-1}	2.0	nd
5 mg ml^{-1}	22.2	nd

These data (together with visual observations) show that whilst the precipitates contain the drug, because of resistance to solubilisation it is relatively difficult to extract it from the amylose matrices and the systems are potentially very useful for the slow and controlled release of drugs.

Retrograded Amylose Mixtures

Regardless of true complex formation, it appears that retrogradation of α-glucans in the presence of therapeutic molecules has the potential to be used as a novel drug delivery system. This system might be adapted whereby retrograded amylose is mixed directly with a therapeutic agent and then dried.

To demonstrate the potential for this system to work, retrograded amylose gel was produced according to the procedure described above (5 ml of 30 mg ml^{-1} amylose dissolved in DMSO, 40 ml distilled water at 85 °C and 5 ml methanol with no solubilised drug mixed in a small flask and refrigerated for 30 minutes). After this time, the contents of the flasks were mixed and poured into 70 ml centrifuge tubes and centrifuged at 3000 rpm (1,500 x g) for 5 minutes. The supernatant was decanted. To the wet gel, aspirin (125 mg to about 150 mg α-glucan) was added and the material was thoroughly mixed. The drug could have been introduced by pre-dissolving in any appropriate solvent. The material was air or freeze dried. The material that was produced using this procedure

was a very rigid gel matrix. Initial attempts to solubilise aspirin from the matrix in water proved very difficult, thereby providing basis that this matrix could be used as the basis for slow release of chemical moieties.

The retrograded amylose-drug association can (and this is especially appropriate for aspirin in view of its high melting point) be heated to circa 150°C to melt the retrograded amylose and enhance entrapment of the aspirin (or other moiety) within the polysaccharide matrix when re-cooled to room temperature. The system is applicable to other α-glucans than the amylose used here.

Whilst retrograded amylose is very indigestible by the digestive enzymes of man, it is recognised that retrograded amylose is hydrolysed and metabolised by the microflora within the colon. It is suggested that this approach is used when drugs are targeted at the colon or for agents (e.g. insecticides, fertilisers) might be appropriate for very slow release in the environment.

Complex Formation

It is suggested that although ibuprofen may have a structure which is more conducive to complex formation than the other molecules described here, many molecules could complex if other conditions were favourable. Melting points of the drugs are probably key characteristics which contribute to favourability of complexing. For the ibuprofen, the melting point of c. 52°C means that it is more mobile in the 85°C systems described here, whereas for the other molecules higher temperatures which are closer to their melting points would favour complexing.

Experimentation will provide appropriate conditions to facilitate complex formation with all the compounds described here and other chemical moieties. It is likely that whilst the DMSO facilitates solubilisation of the amylose, it has a detrimental effect on complex formation and more appropriate solutions would be more productive. However, this work does indicate that there is strong potential to use this approach as a drug delivery system.

CLAIMS

1. A composition comprising mechanically damaged starch and one or more active ingredients.

2. A composition as claimed in Claim 1 wherein the mechanically damaged starch is characterised in that it is capable of forming a gel and/or soluble material on hydration.

3. A composition as claimed in Claim 1 or Claim 2 wherein the mechanically damaged starch is characterised in that it includes amylopectin of degree of polymerisation of DP 2000 to 60.

4. A composition as claimed in Claim 1, 2 or 3 wherein the mechanically damaged starch is characterised by the presence of stress cracks and/or fractures.

5. A composition as claimed in Claim 1, 2, 3 or 4 wherein the mechanically damaged starch is characterised by the presence of reduced crystallinity as compared to native starch.

6. A composition according to any one of Claims 1 to 5 where the mechanically damaged starch is in the form of native granules or a powder.

7. A composition according to any one of Claims 1 to 6 where the mechanically damaged starch has a level of damage of 30 to 80%.

8. A composition according to any one of the preceding claims for controlled, delayed or targeted release of the active compound or material.

9. A composition according to any one of the preceding claims where the active compound or material is selected from aspirin and ibuprofen.

10. A composition according to any one of the preceding claims which further comprises one or more auxiliaries.

11. A composition according to Claim 10 where the auxiliary is a carboxymethylcellulose.

12. A method for the preparation of a composition according to any one of Claims 1 to 11 comprising admixing damaged starch with one or more active compounds or materials.

13. Use of mechanically damaged starch as an excipient.

14. Mechanically damaged starch for use in therapy.

15. A composition comprising partially gelatinised starch and one or more active compounds or materials.

16. A composition as claimed in Claim 15 characterised in that the starch retains some of its native semi-crystalline character.

17. A composition according to Claim 16 where the partially gelatinised starch is native, modified or mechanically damaged starch.

18. A composition according to Claim 15, 16 or 17 where the partially gelatinised starch is in granular form or a powder and the active material is selected from one or more

19. A composition according to any one of Claims 15 to 18 for controlled, targeted or delayed release of the active compound or material.

20. A method of preparation of a composition according to any one of Claims 15 to 19 comprising admixing partially gelatinised starch with one or more active compounds or materials.

21. Use of partially gelatinised starch as an excipient in a controlled, delayed or targeted release formulation.

22. Use of partially gelatinised starch in the preparation of a controlled, delayed or targeted release formulation for use in therapy.

23. A composition comprising a linear alpha glucan and one or more guest molecules wherein the guest molecule is complexed with the helices of the alpha glucan.

24. A composition according to Claim 23 wherein the alpha glucan is amylose.

25. A composition according to Claim 23 or Claim 24 wherein the alpha glucan is native, modified or mechanically damaged starch.

26. A composition according to Claim 25 in which the starch is in granular form or a powder.

27. A composition according to Claim 26 wherein the guest molecule is selected from one or more of aspirin, folic acid, ibuprofen, retinol acetate and β-carotene.

28. A method for the preparation of a composition according to any one of Claims 23 to 27 comprising admixing a solution containing one or more alpha glucans with one or more active materials.

29. A method according to Claim 28 wherein the mixture is heated at a complexing temperature.

30. Use of an alpha glucan as an excipient for controlled, delayed or targeted release formulations.

31. An alpha glucan for use in therapy.

32. A method of therapy comprising administering a composition according to any one of Claims 1 to 11, 15 to 19 or 23 to 27 to a patient requiring therapy.